5 Challenge of Food and the Environment

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5.1 ROLE OF FOOD HETEROGENEITY

Foods are typically not homogeneous. The structure of the food creates local chemical or physical environments that affect the spatial distribution of microorganisms

TABLE 5.1
Examples of the Heterogeneity of Foods

Structure of the Food	Examples of Food	Model Experimental Systems Used to Mimic This Food Structure
Liquid	Soups, juices (with some suspended material)	Broth culture medium
Gel	Pate, jellies, skimmed milk cheeses, such as cottage cheese	Cells immobilized in agar or gelatin (including in a specifically designed Gel Cassette System)
Oil-in-water emulsion	Dairy cream, milk, salad cream, mayonnaise	Alkane:culture medium emulsions
Water-in-oil emulsion	Butter, margarine, low fat spread	Culture medium:alkane emulsions
Gelled emulsion	Whole milk cheese	Alkane:culture medium emulsions, where the aqueous phase is gelled with agarose
Surface	Vegetable tissues, meat tissues	Agar or gelatin (including a modified version of the Gel Cassette System)

as well as their survival and growth.[197] Microorganisms occupy the aqueous phase of foods, and structural features of this phase (Table 5.1) relevant to the length scale of microorganisms can influence their growth. The effects of these structural features on microbial growth include constraints on the mechanical distribution of water,[77,78] the redistribution of organic acids, including those used as food preservatives,[31,32] and constraints on the mobility of microorganisms.[30,60,61,109,110,139,152,153,201]

Many foods will contain a number of microstructural features, and the behavior of microorganisms is influenced differently in each. For example, Parker et al.[140] described the effect of microstructure on the distribution and growth of microorganisms in Serra cheese. Some growth occurred in liquid regions, while other microorganisms formed colonies on surfaces and within the protein gel of the curd (Figure 5.1). Predictions based on data obtained from broth systems can be applied successfully to organisms growing in structured foods. However, where the structure of the food results in a different behavior, this is described below, together with model experimental systems for its study. In many cases growth is "fail-safe," in that organisms grow more slowly in structured systems than in broths. Wilson et al.[197] suggested that this may explain the differences that food manufacturers sometimes observe, where challenge testing of real foods indicates growth at a slower rate than suggested from predictive models. Additionally, the complexity of food structure has been identified as a major contribution to the "overall error" included in microbiological modeling predictions.[144]

5.1.1 Aqueous Phase

Growth in a liquid aqueous phase is typically planktonic, with motility allowing taxis to preferred regions of the food. Diffusive transport of nutrients to

a)

b)

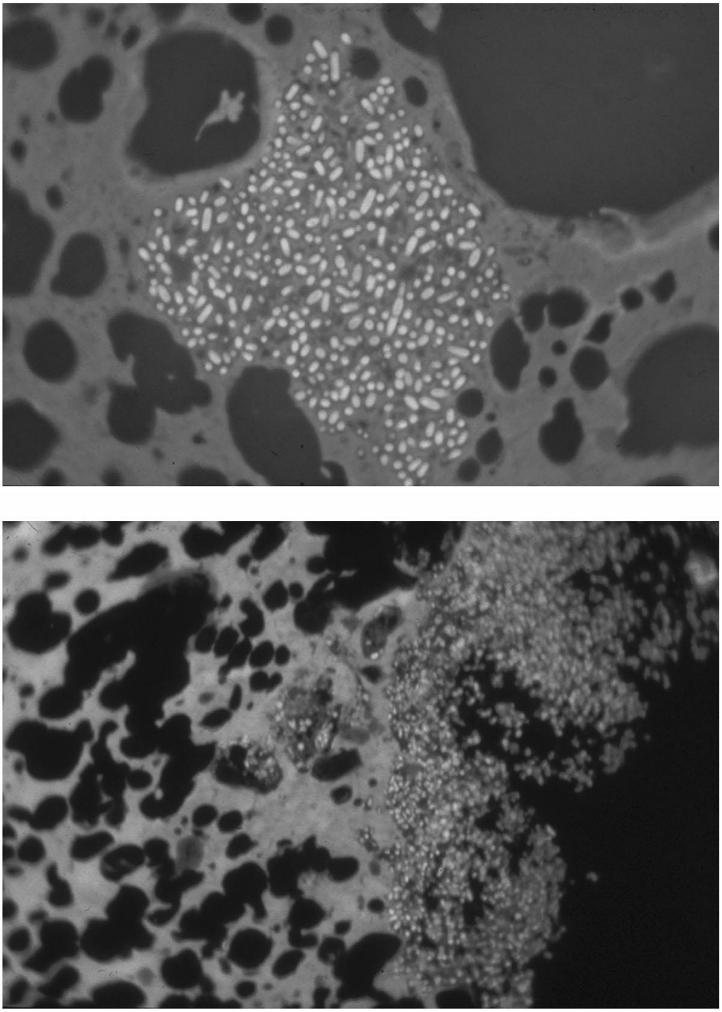


FIGURE 5.1 Light micrographs demonstrating some structural heterogeneity in hard cheese, and showing (a) a colony embedded within the gelled protein of the cheese curd and (b) a colony growing on the surface. The black irregular shapes are embedded globules of milk fat.

microorganisms and of their metabolites away can result in a locally stable equilibrium environment until accumulation of microbial biomass and metabolites cause bulk chemical changes. This is typically manifested by changes in pH or in gaseous composition. When broth culture medium is used in microbiological experiments it is this environment that is mimicked, and, with few exceptions, models for bacterial growth and death have been developed in such simple broth systems. The complexity of foods has been recognized for many years, and it has been suggested that the development of detailed models to account for all aspects of microbial growth in foods may be too costly, and will not yield useful

intermediate models.[14] Simplifying assumptions can be made, and models derived in this way have proved useful.[14]

However, with improving knowledge and the advent of mechanistic modeling approaches it is possible to make predictions of the behavior of microorganisms in structured foods.

5.1.2 Gelled Aqueous Phase

In gelled regions microorganisms are immobilized. This can occur as single isolated cells, or when these multiply, they are constrained to grow as colonies.[60,61,84,88,140,201]

Model experimental systems for studying colonial growth include agar[17,124] and gelatin in a specifically designed Gel Cassette System.[28] Immobilized growth as colonies results in local depletion of oxygen[200,201] and local accumulation of end-products of metabolism, which results in a local decrease in pH within and around the colonies.[104,192,201] Immobilized bacteria also differ from planktonic cultures in their susceptibility to antimicrobial compounds, their energy metabolism, and their metabolic end-products.[165,193] Accordingly, in gelled regions of foods, the growth of microorganisms will result in local changes in the concentration of their growth requirements and metabolites. This results in growth at a slower rate and to a lower yield than planktonic, or free-living cells.[30,152] A unifying theory of microbial growth, which includes proposed equations for a structured-cell mathematical model, influences of local environmental conditions on growth, influences of the microorganisms themselves on the environment, transport of solutes between phases, and physical expansion of colonies,[152] has been developed to attempt explanation of these growth characteristics.[79] Experimental data demonstrate both a decrease in growth rate and shrinkage of habitat domain in the case of *Listeria monocytogenes*, *Listeria innocua,* and *Bacillus cereus*. In all of these cases, the use of a predictive model based on data from the broth experiments would lead to a "fail-safe" prediction in the gelled system. However, Wilson et al.[197] described the growth of *Staphylococcus aureus* as a function of sucrose concentration. In the absence of sucrose, growth was slower than in the broth cultures when the cells were immobilized in gel. However, as the concentration of sucrose was increased, the growth rate in broth decreased, but remained unaffected in gel. Hence, these authors identified conditions of a concentration of sucrose above ca. 15% (w/v) at pH 6 where growth was faster in the case of cells immobilized in gel than for cells in broth (i.e., "fail-dangerous" if a model prediction was based on data from broth cultures).

Growth of cells immobilized in gelatin has been examined under nonisothermal conditions.[28] This study showed that immobilized cells differ from planktonic bacteria during temperature cycling when stressed by high salt or low pH. A finite-difference scheme has been used to combine thermal inactivation modeling with thermal conduction modeling to simulate inactivation of bacteria immobilized within agar blocks.[17]

The local accumulation of metabolic end-products within and around colonies can result in interaction between them. Such competition resulting from close spatial distribution has been termed propinquity, and occurs up to a separation distance of between 1400 and 2000 μm.[177,201] The authors of these works go on to emphasize

that a gap exists between model systems and food, and that to bridge it requires the combined efforts of food microbiologists and microbial physiologists.[201]

5.1.3 Oil-in-Water Emulsions

Here, structure is affected by the concentration and form of the oil phase. The concentration of oil in food varies considerably,[32] and in milk is typically between 3 and 5% (v/v), but in mayonnaise may be between 26 and 85% (v/v). The oil phase exists as polydispersed droplets with a mean diameter that is typically between 0.15 and 8 μm. In concentrated emulsions, the space of the interstices between the droplets is of the same order of size, which is also the same order of size as many bacteria.

In model experimental systems a relationship exists between the concentration of oil and the form of growth of microorganisms.[139] Where the concentration of lipid phase was low (30% v/v) the growth of bacteria was as free-living (or planktonic) cells. An increase in the concentration of the oil phase had no effect on the form of growth of bacteria until it was increased to 83% (v/v). Here the bacteria became immobilized between the close-packed oil droplets. This entrapment resulted in growth not as planktonic cells, but as discrete colonies. The droplets within emulsions confer opacity, and hence visualization of microorganisms is difficult. A mixture of chloroform and methanol was used to selectively remove the oil phase and allow the examination of colonies *in situ*.[30,139] The investigators showed that the colonies are formed from a single bacterium, and as they expanded they displaced the emulsion droplets. Immobilization of bacteria by the lipid component and subsequent growth as colonies resulted in a decreased rate of growth and a shrinkage of the habitat domain compared with growth as planktonic cells — essentially, similar results to the consequences of colonial growth in gels.

5.1.4 Water-in-Oil Emulsions

These consist of an internal aqueous phase dispersed as discrete spherical or irregularly shaped droplets within an outer oil phase, which may contain a mixture of fluid and crystalline fats. In the case of margarines the droplets of aqueous phase are typically irregular in shape, and can range between 0.3 and 30 μ in diameter.[186]

Droplets can be contaminated with microorganisms at the point of emulsion manufacture.[186] The proportion of droplets occupied by microorganisms is small, and a model to predict microbiological contamination based on a function of the initial contamination, and the numbers of droplets exceeding the minimum size for occupancy, has been developed.[186]

Classical theories to describe microbial growth rely on the maintenance of discrete compartmentalized droplets that restrict the availability of water, space, and nutrients for growth. On the basis of these assumptions, Verrips and Zaalberg[186] and Verrips et al.[187] used a mechanistic approach to predict the growth of bacteria within discrete droplets related to the dimensions of the occupied droplets. This was expanded further by modeling the energy demands of the contained bacteria.[175] Models are useful here to predict states that are difficult to measure, and predictions confirm that bacteria in the droplets can grow well, but that their numbers remain

small when expressed per unit volume of emulsion (although their local number density within a droplet is extremely high). Additionally, microorganisms cease to grow when the concentration of metabolic end-products (typically organic acids) becomes toxic or if a requirement for growth, such as oxygen or a carbon source, is exhausted. Models confirm that bacterial growth is restricted when the food structure remains intact (i.e., when coalescence of the droplets does not occur). This was observed in model experimental systems where an increase in numbers of bacteria in water-in-oil emulsions was always accompanied by coalescence of the droplets of aqueous phase.[31]

5.1.5 Gelled Emulsions

Many food emulsions are gelled. This can occur by the deliberate addition of gums or thickeners to increase the bulk viscosity (such as in sausages) or the denaturation of protein to form protein micelles (such as in cheese). Microorganisms are immobilized and constrained to form colonies much as in gelled systems described above.[60,61,140]

5.1.6 Surfaces

The simplest form of food structure is the surface. Growth of bacteria on the surface of food has been measured on Canadian wieners,[118] pâté,[69] and vegetable tissues.[27] Model experimental systems are numerous and include agar gels,[53,115,168,179,199,202] agar film,[115] two-dimensional gradient plates,[178–180,203,204] and a modification of the Gel Cassette mentioned above.[29]

Nicolai et al.[132] modeled surface growth with the assumption that it was in a surface film of liquid. However, growth on a surface is typically colonial. Hence, constraints on growth are similar to those described in the case of gels. Some key differences are important in modeling. Crucially, diffusion limitations are greater at a surface than within an enveloping gel. This was confirmed by Wimpenny and Coombs,[200] Peters et al.,[141] and Robinson et al.[155] who measured the depletion of oxygen and accumulation of protons immediately beneath the colony and extending into the substratum. Colonial growth on surfaces results in decreased growth rates, and comparisons of the growth rates of *Salmonella typhimurium* affected by increasing salt or sucrose followed the order: broth > immersed colonies > surface colonies.[29] This suggests that the rate of growth on surfaces may not be well predicted by models derived from broth systems.[29] Spatial distribution on a surface leads to interactions between colonies.[176] Spatial and temporal variations have a major influence on the potential of surfaces to support bacterial growth. In foods, it is particularly the availability of water.[50] Drying of a food may be deliberate to inhibit growth, and desiccation of microorganisms has been reviewed.[146] A solid surface model system was developed to study the effect of gas atmosphere on growth of several psychrotrophic pathogens.[21] This system demonstrated that increased CO_2 markedly inhibited the growth of all pathogens. The model system can be applied to examination of the growth of pathogens on minimally processed produce under modified

atmospheres. Radial growth of colonies of *B. cereus* on a solid agar surface was dependent on interaction between agar concentration and water activity.[168]

5.2 MODELING THE FOOD ENVIRONMENT

In order to predict the growth of microorganisms in foods reliably, it is vital to use the correct initial chemical conditions. The structural heterogeneity of foods results in a chemical heterogeneity, which is often complicated by dynamics within the food that create a "new" chemical environment. Models of varying complexity exist that can predict the true initial chemical state of foods. Microorganisms occupy the aqueous phase of foods,[30,184] and hence, it is the chemical composition of this phase that requires accurate prediction.

Many foods rely for their preservation on the concentration of organic acids (e.g., acetic, lactic, benzoic, or sorbic acid). In addition, the concentration of sugars or salts can contribute to preservation. It is, therefore, no surprise that many predictive models use combinations of pH and water activity (although often expressed as concentration of NaCl) together with temperature as the three major determinants of growth. What follows is a summary of available models that can predict the initial environmental conditions within foods.

5.2.1 Organic Acids

Acetic, lactic, benzoic, and sorbic acid (and their salts) are added as preservatives in many foods, although acetic and lactic acids are also produced in fermented foods as end-products of microbial metabolism. Their preservative action is by virtue of a combination of their effect on the pH of the food and the antimicrobial properties of the undissociated form of the molecule. Accordingly, their antimicrobial effect is influenced by the fundamental thermodynamic characteristics of dissociation and partition. It is these that must be modeled to predict the potential of foods to inhibit the growth of microorganisms.

5.2.2 Dissociation

Weak organic acids dissociate (or separate) into their component parts. In the case of acetic acid, this occurs as:

CH_3COOH	$\Leftrightarrow$	CH_3COO^-	and	H^+
acetic acid		acetate		hydrogen ion
(undissociated)		(dissociated)		(proton)

This dissociation is key to prediction of the concentration of the undissociated form of the acid, which has the predominant antimicrobial effect in foods.[11,65,166]

The Henderson–Hasselbalch equation relates the pH of the food to the pK_a and the relative proportions of dissociated and undissociated acid in foods have been predicted[198] as follows:

$$pH = pK + \log_{10} \frac{[acid]_{dissociated}}{[acid]_{undissociated}} \tag{5.1}$$

Rearrangement gives the concentration of weak acid in its undissociated (i.e., microbiologically active) form, $[HA]_{aq}$, given the pH, pK_a, and total concentration of weak acid, $[HA]_T$, as follows:

$$[\mathrm{HA}]_{\mathrm{aq}} = \frac{[\mathrm{HA}]_{\mathrm{T}}}{1 + 10^{(\mathrm{pH} - \mathrm{p}K_{\mathrm{a}})}} \tag{5.2}$$

where $[HA]_{aq}$ is the concentration of undissociated organic acid in the aqueous phase and $[HA]_T$ is the total concentration of organic acid. pK_a is the negative logarithm of the dissociation constant K_a, which is a thermodynamic constant controlling the dissociation equilibrium shown above:

$$\mathrm{p}K_{\mathrm{a}} = -\log(K_{\mathrm{a}}) \tag{5.3}$$

K_a is typically a small number, and published values are available.[205] pK_a varies slightly with temperature, and an empirical equation that predicts this variation has been published[154]:

$$\mathrm{p}K_{\mathrm{a}} = \left(\frac{A}{T}\right) - B + (CT) \tag{5.4}$$

where T is the temperature in Kelvin (K), and A, B, and C are shown in Table 5.2. Such predictions are important preliminaries in dealing with the challenge of food and the environment. Without such knowledge it is quite simple to apply an incorrect initial environmental condition when using predictive microbiology tools, and this can easily result in erroneous predictions.

Predictions must also be reiterative. For example, once dissolved, the organic acid will dissociate depending upon local pH, but will then perturb this pH. The dissociation is also dependent on local buffering capacity of the food, and this is

TABLE 5.2
Values of *A*, *B*, and *C* to Be Inserted into Equation 5.4 for Calculation of the Effect of Temperature on pK_a[154]

Acid	*A*	*B*	*C*
Acetic	1170.48	3.1649	0.013399
Lactic	1286.49	4.8607	0.014776
Benzoic	1590.2	6.394	0.01765

extremely difficult to predict. However, Wilson et al.[198] developed a method for performing calculations describing the reiterative dissociation of organic acids, and hence predicting the true chemical composition of foods. This not only allows microbial growth models to predict growth, but also allows the changes in pH caused by microbial metabolism to be predicted. These authors used a theory describing the behavior of weakly dissociating systems, and knowledge of dissociation constants and concentrations. They make the point that food is too complex for solutions to be achieved through complex calculation. Hence, the authors characterized the buffering behavior of food by a titration with a strong (i.e., completely dissociating) acid, and then used knowledge of the dissociation constants of weak acid preservatives to predict the behavior of these in the food. Their calculation scheme may also be applied to a mixture of weak acids including polyacid species such as the tricarboxylic acids (e.g., citric acid).[198]

5.2.3 Partitioning into Oil Phases

In biphasic foods, which contain aqueous and lipid phases, the antimicrobial undissociated acids partition between the aqueous and lipid components.[32] This decreases the concentration of undissociated acid in the aqueous phase. Partition coefficients of acetic, lactic, and sorbic acids between sunflower oil and water have been reported as 0.02, 0.033, and 2.15, respectively,[32] demonstrating the potential for, particularly, the undissociated form of sorbic acid to decrease in the oil phase of biphasic foods.

As a complication, the pH of foods preserved using organic acids is typically in a region where weak organic acids are present in both the undissociated and the dissociated forms. Calculation of the residual concentration of the undissociated form following partition is thus difficult because the concentration is subject to the effects of partition, and to the dissociation equilibrium based on the new pH of the system and the new residual concentration of undissociated acid.

A modified form of the Henderson–Hasselbalch equation has been developed,[198] which takes these effects into account and gives the proportion of the total weak acid in a two-phase system that is present in its undissociated form in the aqueous phase, given the pH, the volume fraction of oil, and the partition coefficient for the undissociated weak acid. It was cast as:

$$\frac{[\mathrm{HA}]_{\mathrm{aq}}}{[\mathrm{HA}]_{\mathrm{T}}} = \frac{1}{1 + K_{\mathrm{P}}\left(\dfrac{\phi}{1-\phi}\right) + 10^{(\mathrm{pH}-\mathrm{p}K_{\mathrm{a}})}} \tag{5.5}$$

where K_P is the partition coefficient and ϕ is the fraction volume of the oil phase. Predictions have been validated in aqueous and biphasic foods.[198]

5.2.4 Water Activity

Water activity (a_w) is a measure of the concentration of available water in a food and can be defined as the tendency of water to escape from a solution relative to its

ability to escape from pure water at a specific temperature. Water activity is equal to the equilibrium relative humidity divided by 100. Pure water has an a_w of 1.000, and an environment where water is absent has an a_w of 0.000.[49,182] Most microorganisms require a high a_w for growth, and a_w is included in many predictive microbiology models. The a_w of foods can be adjusted by the addition of solutes (humectants), such as sodium chloride, sucrose, or glycerol. In some cases, the solute itself may have toxic effects, and the inhibition of growth of microorganisms when sodium chloride is used to adjust a_w can be greater than when glycerol is used, due to the toxicity of high concentrations of sodium chloride.[12,75,182] Care must be taken, therefore, to use only those predictive models that use the same humectant as the food of interest. Prediction of the initial a_w of the food can be achieved from first principles using a variety of equations, such as Raoult's law,[49,93] which was derived by Christian[49] as:

$$\text{Log}_e\, a_w = \frac{-vm\phi}{55.51} \tag{5.6}$$

where m is the molal concentration of the solute, v is the number of ions generated by each molecule of the solute, and ϕ is the molal osmotic coefficient. Commercial software to predict water activity from a list of food ingredients in a recipe is available (e.g., ERH CALC™).

5.3 HURDLE CONCEPT

Hurdle technology involves the use of combinations of physical or physicochemical preservation techniques at subinhibitory levels to control the growth of food-borne microorganisms.[98] This has the effect of conferring microbial safety and stability while maintaining acceptable nutritional and sensorial attributes,[160] an approach that is important for minimally processed extended shelf life foods.[108] With the development of new food products that depend on multiple barriers to ensure safety, it becomes necessary to develop the means to apply predictive microbiology to hurdle technology.[43,97] Careful definition of the conditions defining the boundaries of growth or survival will allow industry to design foods with the appropriate level of safety;[149,160] however, there have been few attempts to provide a quantitative assessment of hurdles.[160]

Examples of interactions include CO_2, pH, and NaCl on *L. monocytogenes*;[71] temperature, pH, citric acid, and NaCl in reduced calorie mayonnaise on *Salmonella* spp.;[121] pH, acid, and salt on *Staphylococcus aureus*;[64] salt, pH, and nitrite on *Escherichia coli* O157:H7 in pepperoni;[151] temperature and pH on *E. coli* O157:H7 in Lebanon bologna;[66] and nisin and leucosin on *L. monocytogenes*.[138]

While it is clear that combinations of hurdles can influence food-borne microorganisms, it is not clear to what extent these factors interact. When the square root model is used to describe the effect of several hurdles such as temperature, pH, and a_w, these factors are usually considered to act independently, with no interactions.[119] Ratkowsky and Ross[149] described a combined probability/kinetic model for *Shigella*

flexneri in which temperature, pH, a_w, and nitrite were shown to act individually. It would be expected, however, that interactions must occur between certain hurdles. For example, interactions between organic acids and pH would be expected (due to the influence of pH on the extent of dissociation as described in Section 5.2) and have been observed.[38,121,147] Effects on heat resistance of *E. coli* due to the interactions between combinations of temperature, pH, NaCl, and sodium pyrophosphate have been modeled.[86,87]

Polynomial models can be used to describe interactions between a wide variety of hurdles. This is because the regression methods used facilitate the search for quadratic or interactive effects. Combination effects have also been modeled using Belehradek and Arrhenius models.[90,91] The growth of *L. monocytogenes* at 9°C as influenced by sodium nitrite, pH, sodium chloride, sodium lactate, and sodium acetate has been modeled,[130] and predictions compared with the growth of organisms in real sausage and predictions from Food MicroModel. Food MicroModel is a software package developed in the U.K. that contains secondary models of the effects of environmental factors (mainly pH, concentration of NaCl, and temperature) on the survival, growth, and thermal death of major food-borne pathogenic bacteria in broth. Predictions were on average within 20% of the Food MicroModel predictions based on 10 experiments although predictions of growth in sausage were, on average, 16% below the observed values based on inoculation of four sausages. This is perhaps related to the effects of structure as described in Section 5.1. The effect of previous growth temperature, previous cell concentration, and previous pH on the lag time and specific growth rate of *Salmonella typhimurium* has been investigated using response surface models.[135–137] In all cases the previous growth history did not influence the predictions of the model.

Some authors contend that predictive models of the combined effects of temperature and water activity and the combined effects of temperature and pH suggest that the effect of the combinations on growth rate is independent.[120] However, these authors go on to state that the factors are interactive at the no-growth interface (i.e., the point where growth ceases). Such interface models quantify the probability of growth and define conditions at which the growth rate is zero or the lag time is infinite. Such new growth interface or habitat domain models have been published.[116,181] Square root models and response surface models were developed to look at the effects of interactions between dissolved carbon dioxide and water activity on the growth and lag time of *Lactobacillus sarcae*.[58] The response surface models showed the best correlation although at low water activities, predictions were illogical. Both models, however, proved to be useful in the prediction of the shelf life of meat products, and were validated by comparison with an existing model.[196] Similarly, a quadratic response surface model was built to predict the combined effects of temperature and modified gaseous atmosphere on the growth of *Yersinia enterocolitica*.[143]

Predictive models have been used to predict the response of *Listeria monocytogenes* exposed to acid, alkaline, or osmotic shock at the time of inoculation on the subsequent effects of temperature, concentration of NaCl, and pH.[47] The authors found that predictive models were unreliable, highlighting potential problems of variable conditions, but failing to consider the implications of adaptation of the

organisms to osmotic or pH effects. An important development is the use of the gamma concept, which assumes that the effects of controlling variables can be multiplied and that the cardinal parameters of temperature, pH, and water activity are not a function of other variables.[196] Accordingly, these authors developed a model based on the prediction of growth rate as a function of temperature and water activity and another where growth rate was predicted as a function of temperature and pH. The two models were multiplied to produce one overall model, which was validated against new experiments. Additive interaction between inhibitors has been observed.[24] These authors used a response surface methodology to model the response of *L. monocytogenes* to a bacteriocin (curvaticine) and sodium chloride: the model showed that the combination of the two inhibitors was greater than the effect of each individually. Interactions between inhibitory compounds were also investigated[8] by using a series of secondary models[7] describing independently the effects of environmental factors.[8] The authors of the latter work then went on to show that, by taking into account interactions between environmental factors, the model decreased the frequency of fail-safe growth predictions from 13.5 to 12.1%, while the frequency of fail-dangerous no-growth predictions decreased from 16.1 to 7.1%. These findings suggest that interactions are occurring within the system, and that the models were taking them into account.[8] However, even with multiplicative models the predictions are less accurate to describe lag time and growth rate near the limits of growth of microorganisms,[7] and lag time models were particularly vulnerable to error.

Inactivation modeling is less common in response to a combination of hurdles. Death kinetics as a function of pH, storage temperature, and concentration of essential oil have been described using a quadratic function, and used to predict successfully the death of *Salmonella* in home-made salads.[89] A regression model describing the heat inactivation of *L. monocytogenes* was based on the Gompertz Equation.[48] The equation enabled separate characterization of the parameters of the shoulder, the maximum slope, and the tail. Interactive effects were then derived from the regression model. This showed that the shoulder region of the survival curve was affected by pH, and the maximum slope by temperature, fat content, and interaction of temperature and milk fat. Model validation was successful for temperatures only above 62°C, however. The combined effects of pH and ethanol on the heat inactivation of *B. cereus, S. typhimurium,* and *Lactobacillus delbrueckii* were modeled using a series of second-order polynomial equations to describe variations in D values resulting from changes in pH or added ethanol.[45] The heat inactivation of *B. cereus* spores was modeled using a new concept of z-value modeling using a z(pH) value,[96] where z(pH) was defined as the difference in pH from a reference pH value required to effect a 10-fold reduction in the D value. A linear relationship between the calculated z(pH) value and the lowest of the pK values of organic acids used to effect heat resistance was found. The heat resistance of *Listeria monocytogenes* in logarithmic phase cells that had been heat shocked at 42°C for 1 h and subcultures of cells that were resistant to prolonged heating has been modeled.[9] A better fit for the survivor curves was found using sigmoidal equations compared with the classical log-linear models. Comparisons between models showed that an increase of thermal tolerance was induced by sublethal heat shock or by the selection of the heat-resistant

population. Both isothermal and nonisothermal heat inactivation effects on the germination and heat resistance of *B. cereus* spores have been modeled.[70] An inactivation model was developed for *Salmonella enteritidis*.[96] It modeled the response of the organism to a range of concentrations of oregano essential oil and temperatures at two pH values. Quadratic functions were then used to predict the growth of this organism in home-made salads. The inactivation kinetics of *E. coli* O157:H7 were modeled using the Baranyi model (based on a set of nonautonomous differential equations)[13] as a function of time to estimate the kinetic parameters.[164] Quadratic models were then developed with natural logarithms taken of the shoulder and death rate as a function of temperature, pH, and concentration of oregano essential oil. The predicted values from the model were validated using viable count measurements made within real salads.

Modeling spore responses (other than inactivation) is unusual. The germination kinetics of spores of proteolytic *Clostridium botulinum* 56A as a function of temperature, pH, and concentration of sodium chloride have been modeled.[46] The germination kinetics were collected and expressed as the accumulated fraction of germinated spores with time and each environmental condition, and this accumulated fraction was then described by an exponential distribution. Quadratic polynomial models were developed by regression analysis of the exponential parameter and the extent of germination as a function of the variables under study. Validation experiments confirmed that the predictions were acceptable, and in most cases were fail-safe.

5.4 COMPETITION WITH OTHER MICROORGANISMS

Existing published models include a wide range of environmental, physical, or chemical factors; however, the competitive influence of microorganisms has not yet been incorporated into them. Competition may not be an issue in many foods, since interactions would not be expected until cell numbers had reached a potential hazard or caused spoilage.[160] On the other hand, growth of *L. monocytogenes* in dairy products is influenced by the natural microflora, and interactions may be difficult to model.[33] Therefore, it has been suggested that competition must be considered in the development of predictive models.[163]

Competition between microorganisms in a solid matrix such as food depends to a large extent on proximity of colonies to each other.[201] Cells growing on surfaces generate gradients of redox potential, pH, oxygen concentration, and nutrients, which can influence the growth of neighboring colonies. This phenomenon can be observed in foods, for example, where "nests" of lactic acid bacteria in fermented sausage influence the survival of food-borne pathogens,[201] and also in dairy products where interactions between natural microflora and *L. monocytogenes* are influenced by the nature of the food matrix.[33]

A related concept is the idea of "maximum carrying capacity" of a food product,[160] in which inhibition of pathogens by other microorganisms takes place when the competing flora have reached numbers at which the environment can support no further growth. This was observed with cocultures of *L. monocytogenes* and *Carnobacterium piscicola*.[35] In this study, the maximum population density of *L. monocytogenes* was reduced by the competing lactic acid bacteria, and this was

attributed to nutrient depletion. It is by no means clear to what extent competition is related to depletion of nutrients. The thermal tolerance of *S. typhimurium* was enhanced by the presence of competing microflora, and it was suggested that the presence of competitors may have influenced the pathogen to induce stationary-phase gene expression.[62]

The interaction of spoilage microorganisms has recently been quantitated by Pin and Baranyi.[142] Polynomial models were developed for a number of microorganisms, and the growth of groups of strains was compared individually and in the total mixture. This approach allowed the identification of the dominant group on the basis of its growth rate and lag time. These authors also showed that reduced growth rate could be attributed to microbial interactions. Competition from naturally occurring microflora has been documented.[94] Here, predictions of the growth of *Pseudomonas* and *Listeria* in meat were made. Predictive models worked well in predicting the growth of both organisms in decontaminated meat and in decontaminated meat inoculated with each organism, together or individually. However, the presence of naturally occurring microflora in non-decontaminated meat prevented the initiation of growth of *Listeria* and the predictive models failed.

A related aspect of interaction is that of the potential for quorum sensing between microorganisms.[101] At low inoculation concentrations, modifications to modeling approaches were necessary to take into account inoculum size variation. Modeling the effects of inoculum size stochastics, however, confirmed that the growth rate was independent of inoculum concentration but that variability occurred as the inoculum concentration decreased.[209,210]

5.4.1 Interactions Based on the End-Products of Metabolism of One Species

This is a complex modeling task, but stoichiometric modeling can be used to relate the end-products of metabolism to the inhibition of the same or an accompanying organism. It assumes a "reaction scheme," and seeks to choose the simplest representation of a system that embodies the behavior of interest.

Thus, a stoichiometric model can predict the local changes in weak acid concentration resulting from microbial growth. This must then be used to predict changes in local pH. This can be done by an empirical characterization, merely by using a titration of the growth environment with the acid of interest, and fitting a curve to these data. Alternatively a quasi-mechanistically based approach may be taken,[132] or use made of a Buffering theory[198] described in Section 5.2. An advantage of the latter is that the model may be easily applied to systems of differing buffering capacity, and can combine the effects of mixtures of weak acids. Diffusion is an integral part of such modeling, and a standard model of Fickian diffusion using published diffusion coefficients in aqueous solution is usually appropriate.

For growth in liquid systems, a cardinal growth model has been combined with cardinal pH data.[99] Cardinal models use the cardinal values (minimum, optimum, and maximum values) of the environmental factors that constrain growth. Instantaneous growth rates from this model were used in a modified Baranyi growth model,[13] together with stoichiometric parameters determined from bioreactor experiments.[197]

The change in pH from production of lactic acid was determined by use of a Buffering theory.[198] Very close agreement was found between the model and the data.

5.4.2 Mixed Culture

Application of stoichiometric approaches to mixed cultures also works well. Wilson et al.[197] showed the growth of a mixed culture of *Lactococcus lactis* and *Listeria innocua* in a bioreactor at pH 4.5. Predictions used cardinal model parameters,[99] and stoichiometric parameters from bioreactor experiments.[197] A Buffering theory[198] was used to predict changes in pH. Such an approach provided good prediction of both the rate and extent of growth of the two organisms. Of interest in these approaches is that a stationary phase was not incorporated into the primary growth model, but emerged from the prediction in response to the accumulation of metabolites.

Interactions resulting from the production of antimicrobial bacteriocins by lactic acid bacteria in conjunction with the inhibition resulting from production of lactic acid have been modeled.[44] These authors used a modification to logistical equations that described the combined (although not additive) effects of two or more inhibitory compounds. They then applied their findings to the inhibition of *Leuconostoc mesenteroides.* The inhibition of growth of *Enterobacter cloacae* by *Lactobacillus curvatus* resulted from the production of lactic acid by the latter, and the concomitant decrease in pH,[105] which was also inhibitory to *L. curvatus.* This interaction has been modeled using a set of first-order differential equations describing growth, consumption, and production rates for both microorganisms.[107] Parameters were obtained from pure culture studies and from the literature, and the equations were solved using a combination of analytical and numerical methods. Predictions of growth of mixed cultures used parameters from pure culture experiments, which were close to the experimental data. The models also showed that interactions occurred when the antagonistic bacterium, in this case *L. curvatus*, reached 10^8 cfu/ml.

5.5 ADAPTATION AND INJURY

5.5.1 Effects of Environment on Adaptation

Predictive microbiology should deal with bacterial stress within populations.[6] An example is the extension of the lag time of *Listeria monocytogenes* under suboptimal conditions when the inoculum was stressed.[6] More important, considerable interest has arisen recently in the problems of adaptive responses of bacteria and in the cross-resistance that this can confer. For example, adaptation of bacteria to methods of preservation can result in survival or growth that is better than predicted if the adaptive response is ignored. Accordingly, adaptation of bacteria can lead to unsafe or spoiled food.[34] The implications of adaptation can be demonstrated by reference to the acid tolerance response (ATR). The ATR in *L. monocytogenes* has been attributed to the *de novo* synthesis of proteins (sometimes referred to as acid shock proteins) when exposed to a decrease in extracellular pH.[134] Such biochemical changes confer acid resistance on the organisms, but O'Driscoll et al. also noted

that *L. monocytogenes* that had been induced to show the ATR also had an increased resistance to thermal, osmotic, and cold stresses.[134] ATR has been defined as the resistance of cells to low pH when they have been grown at moderately low pH or when exposed to a low pH for some time,[59] and is typically demonstrated in broth culture, where a pH of 4.8 to 5.0 is reported to give an optimum ATR.[56] Many foods fall into this region of pH, and, more important, many microorganisms can experience this pH transiently during food production or sanitation protocols. Adapted populations could then result.

Additionally, it is clear from the above sections that one of the key effects of food structure is the immobilization of microorganisms and their resultant growth as colonies. This results in local changes in the concentration of substrates[201] and, particularly, a local accumulation of acidic metabolic end-products leading to a decline in pH within and around the colony[104,192] with a pH gradient extending into the surrounding menstrum.[192,201] In the case of *S. typhimurium*, the pH gradient extended from the original pH 7.0 in the surrounding medium to pH 4.3 inside the colony.[192] Such a local decline in pH within the colony is greater than the change required to stimulate an ATR in *Salmonella* and other Gram-negative enteric bacteria[95] and in *L. monocytogenes*.[92] It is conceivable, therefore, that cells of food-borne pathogenic bacteria immobilized as colonies embedded in a food matrix may undergo a self-induced ATR stimulated by a localized pH that has declined by virtue of the colony's own metabolic processes. It is known that acid shock proteins are synthesized and exported from cells experiencing adaptation in broths. Should this also be the case in colonies, it would result in cells within the colony becoming acid tolerant.

Despite the importance of adaptation in food microbiology, attempts to model it are rare. Authors have acknowledged that organisms behaved differently when exposed to changes in pH or sodium chloride concentration, and that exposure to these agents during exponential phase had a more dramatic effect than during the lag phase when adaptation was possibly induced.[47] However, no attempt to incorporate adaptive responses into models was made. A cross-resistance between high hydrostatic pressure and mild heat, acidity, oxidants, and osmotic stresses was demonstrated for *E. coli* O157.[20] Differences were most dramatic in stationary-phase cells; the only exception being acid resistance where differences were also apparent in the exponential phase, although, again, no attempt to incorporate these into a model was made. In one attempt to model adaptation, a model to describe the influence of temperature and the duration of preincubation on the lag time of *L. monocytogenes* was developed.[10]

5.5.2 Effects of Sublethal Injury

Subjection of bacteria to inimical processes can result in the cumulative injury of the bacteria, resulting in death. Sublethal injury is the reversible damage inflicted on bacteria that is insufficient to cause a loss of viability, and from which the bacteria can recover.[5,80,150] It is an important phenomenon to recognize when collecting data for modeling, because bacteria can often fail to form colonies on conventional selective microbiological culture medium used for their enumeration.[2,127] They can

also fail to respond positively to viability stains.[26] However, the cells can remain viable and the injury can be repaired in foods, where the bacteria can then increase in numbers.[82,206] The severity of treatment that results in sublethal injury differs between species, although serotypes of *Salmonella* have been found to respond similarly to one another.[128]

5.5.2.1 Enumeration of Sublethally Injured Bacteria

A range of methods have been used to determine the extent of injury of microorganisms. These include differential plate counts on selective and nonselective agars[15,150] or on minimal and more complex media,[102] extension of the lag phase,[4,102] and changes in bioluminescence.[67] Such methods can be used to optimize both the recovery medium and the time and temperature of incubation. For example, it has been shown that cells of *L. monocytogenes* that were subjected to sublethal injury by heat exhibited a broad optimum temperature for recovery, with an optimum between 20 and 25°C, but that incubation at 2 or 5°C failed to allow repair.[103] The time taken for repair of injury to complete can be determined by measuring the time before equivalent counts are found on a selective medium (which will not support the growth of sublethally injured bacteria) and a nonselective culture medium (which will allow the growth of sublethally injured bacteria).[103] Some modeling of resuscitation has been published.[117] Predictions of response might be possible: for example, a relationship was found between the concentration of sodium chloride in the heating menstrum and its concentration in the growth medium used for the resuscitation and subsequent enumeration of *S. typhimurium*.[106]

5.6 VALIDATION IN FOODS

One of the most important aspects of model development is ensuring that predictions made by the model are applicable to real situations. This is the validation process. It should involve comparisons of the predictions of the model with observed measurements, which should be different data to those used to construct the original model. Although some predictive models have been constructed in real foods (see later in this chapter), the vast majority of models have been constructed from experiments performed in laboratory culture media (typically broth). In all cases the validation process should, ideally, include comparisons with the behavior of microorganisms in real foods or during real food processes. However, due often to cost but also other factors, validation can be done in model systems, or using previously published data. A validated model should be consistently "fail-safe," that is, predictions should fail on the side of safety (i.e., predicted growth rate and lag time should be faster and shorter, respectively, than experimental values). Predictive models can be crucial aspects of HACCP protocols. Imaginary scenarios depicting the way in which predictive models can be incorporated into HACCP concepts have been published,[122] as has a useful review of the application of predictive food microbiology in the meat industry.[113] Similarly, predictive microbiology is an important element of Quantitative Microbial Risk Assessment (QMRA). Models are useful decision support tools, but it should be remembered that models are, at best, only a simplified

representation of reality. The application of model predictions should be tempered with previous experience and with knowledge of other microbial ecology principles that may be experienced in the food by the organism.[158] Sources of data and models relevant to the growth of *L. monocytogenes* in seafood and that could be part of a QMRA have been published.[158]

5.6.1 Bias and Accuracy

Some criticism of the term "validation" revolves around the difficulty in quantifying just how well models perform their predictive role. Error occurs implicitly in the use of data for modeling and the use of those models for the prediction of growth of microorganisms. There are a number of potential sources of error: the homogeneity of foods; the completeness of the environmental factors used to collect the data; conversion of empirical results to a mathematical function; and fitting the models to the data.[159] For example, the overall errors in the application of growth models to the growth of *Pseudomonas* species in food and in laboratory media have been quantified.[144] The authors made the point that the error was small in the case of culture medium but great in the case of food, and went on to quantify the influence of food structure and composition on the overall error. Sutherland et al.[172] found that much of the published work on *E. coli* O157:H7 was done under conditions outside of the experimental values used to develop their growth model. These workers also reported that validation with data from cheeses and meats was difficult because the original authors often did not report experimental conditions such as NaCl content or pH. In these cases, poor predictions were often made. Similar observations were made when a growth model for *B. cereus* was being validated.[170]

It is clear in the above cases that some quantification of the deviation of the predictions from the observed values would be useful. Many measures of such quantification of error in the validation process have been made.[157] Additionally, however, Ross[157] has proposed using simple indices of the performance of models as a step towards an objective definition of the term "validated model." These indices give an indication of the confidence with which those models can be used (accuracy factor), and whether the model displays bias towards fail-dangerous predictions (bias factor). The accuracy factor is defined as:

$$\text{Accuracy factor} = 10^{\left(\Sigma\left|\log(GT_{predicted}/GT_{observed})\right|/n\right)} \tag{5.7}$$

where $GT_{predicted}$ is the predicted generation time and $GT_{observed}$ is the observed generation time, and n is the number of observations. The less accurate the predictions the larger the accuracy factor.

The bias factor is defined as:

$$\text{Bias factor} = 10^{\left(\Sigma\log(GT_{predicted}/GT_{observed})/n\right)} \tag{5.8}$$

If no disagreement between predicted and observed values occurs then the bias factor is equal to 1. However, a value of the bias factor greater than 1 indicates a fail-

dangerous model because it will predict generation times longer than actually observed. It should be noted, however, that when rate values are used to compute the bias factor, a fail-dangerous model will have a bias factor of less than 1.

As mathematical techniques advance, so does the process of comparing models. The use of artificial neural networks has been identified as a useful alternative technique for modeling microbial growth. Neural networks also lend themselves to quantifying comparisons between models and suitable indices have been suggested.[85]

5.6.2 Validation Using Literature Values

The most common method of validation is the use of literature data. This is based on the assumption that if the published experiments were performed under well-defined conditions that do not differ markedly from those used to develop the model, then the model predictions should be reasonably reflected in the published data. A large number of models have been validated using published information including models for *Y. enterocolitica*,[22,100,171] *Aeromonas hydrophila*,[112] *Clostridium botulinum*,[76] *S. enteritidis*[23] and *E. coli* O157:H7,[23,173] *L. monocytogenes*,[71,111] and a number of other microorganisms.[55]

There are, however, some potentially serious limitations to the use of literature data for validation of predictive models. Additional food components are frequently responsible for deviations between predicted and observed values in validation experiments. For example, Tienungoon et al.[181] predicted the growth limits of *L. monocytogenes* as a function of temperature, pH, NaCl, and lactic acid. The authors used two strains of *L. monocytogenes*, Scott A (a pathogenic strain) and L5 (a wild-type strain isolated from cold-smoked salmon). Experiments were carried out in broth culture at a wide range of environmental conditions. Aliquots of the inoculated media were observed for a period of 90 days to determine whether the conditions supported growth. Data from the experimental program were modeled using a probability model for growth. Figure 5.2 shows the growth boundary predicted by the model for the case of no added lactic acid, and a water activity of 0.992 (representing 0.5% NaCl in a typical culture medium) as a function of temperature and pH. This boundary is plotted alongside the data from the literature (Table 5.3). Generally, the model predicted values that were in good agreement with literature values. However, where deviation from the observed measurements occurred, this was usually explained by additional identifiable preservative factors in the system, and these are described in Table 5.3.

A similar issue arises using the growth boundary model of McKellar and Lu,[116] which predicts the growth limits of *E. coli* O157:H7 as a function of temperature, pH, NaCl, sucrose, and acetic acid. These authors used five strains of *E. coli* O157:H7 growing in broth culture for a period of 72 h to determine whether the conditions supported growth. Data from the experimental program were modeled using a probability model for growth.

This boundary is plotted alongside the data from the literature (Table 5.4) in Figure 5.3. As above, the model predicted values that were in good agreement with literature values. Again, however, deviation from the observed measurements occurred, due to additional identifiable preservative factors, which are described in Table 5.4.

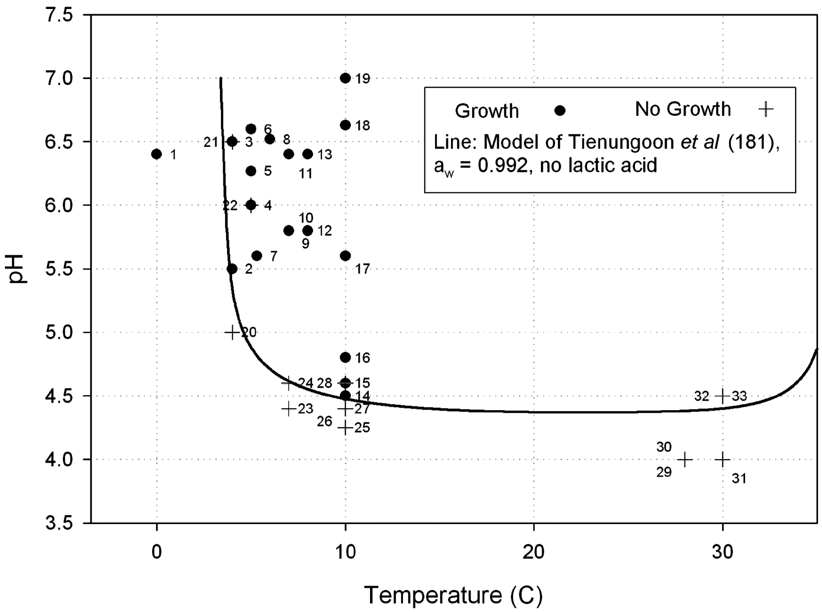


FIGURE 5.2 The growth boundary predicted by the model of Tienungoon et al.[181] for the case of no added lactic acid, and a water activity of 0.992 (representing 0.5% NaCl in a typical culture medium) as a function of temperature and pH. This boundary is plotted alongside the data from the literature described in Table 5.3.

Other sources of error associated with the use of literature data include lack of information on preincubation conditions that might result in the development of acid tolerance; use of selective media for enumerating microorganisms; lack of estimates on variability; and presence of factors in foods that are not taken into account in models (e.g., preservatives).[39] It appears that the most appropriate method for validation might be to use data derived under well-controlled conditions, so that the model's performance will not be unfairly biased.[157] Unsafe predictions and lack of published information on error also limit the usefulness of literature data, and emphasize the need to validate against new data.[57]

5.6.3 Validation in Foods

The most common method for validating models using new data is to carry out experiments directly in the food product of concern. Thus, several models have been validated directly in food products including survival of *L. monocytogenes* in uncooked-fermented meat,[195] fishery products,[158] or pâté;[69] survival of *Campylobacter jejuni* in a variety of foods;[54] growth of *L. monocytogenes* in dairy products;[129] growth of *L. innocua* in Bologna-type sausage;[81] growth of *Staphylococcus aureus* in sterile foods;[190] growth of *E. coli* O157:H7 on raw ground beef;[188] growth of *L. monocytogenes* in sterile foods;[189] growth of *Shigella flexneri* in sterile foods;[207] growth of *E. coli* on raw displayed pork;[73] growth of *Y. enterocolitica* in seafood;[143] and growth of *Listeria* in a range of foods.[174]

TABLE 5.3
Literature Values Used in the Validation of the Growth Boundary Model Shown in Figure 5.2

Data Ref.	Temp. (°C)	pH	Other Hurdles[a]	Matrix[b]	Ref.	Obs. Time[c]
			***Listeria monocytogenes* — Growth Data**			
1	0	6.4		Chicken broth	191	
2	4	5.5	0.5% NaCl	TSBYG	71	
3	4	6.5	4% NaCl	TSBYG	71	
4	5	6	4.5% NaCl	Tryptose phosphate broth	42	
5	5	6.27	0.05% NaCl	Minced beef	111	
6	5	6.6	0.05% NaCl	UHT milk	111	
7	5.3	5.6	0.05% NaCl	Vacuum packed lean beef	111	
8	6	6.52	0.05% NaCl	Chicken legs	111	
9	7	5.8	0.05 [0.004]% acetic acid[d]	Tryptose broth	3	
10	7	5.8	0.05% citric acid	Tryptose broth	3	
11	7	6.4	0.05% NaCl	Nonfat milk	111	
12	8	5.8	0.05% NaCl	Minced beef	111	
13	8	6.4	0.05% NaCl	Skimmed milk	111	
14	10	4.5		TSBYE	181	
15	10	4.6	Poised with citric acid	TSB	167	
16	10	4.8	Poised with lactic acid	TSB	167	
17	10	5.6		Tryptic meat broth	16	
18	10	6.63	0.277% NaCl + 170 ppm nitrite	Vacuum packed ham	111	
19	10	7	a_w = 0.96	Tryptic meat broth	16	
			***Listeria monocytogenes* — No Growth Data**			
20	4	5		TSBYG	72	28 d
21	4	6.5	8% NaCl	TSBYG	71	70 d
22	5	6	4.5% NaCl + nitrite	Tryptose phosphate broth	42	NS
23	7	4.4	0.2% citric acid	Tryptose broth	3	400 h
24	7	4.6		TSBYG	72	28 d
25	10	4.25		TSBYE	181	NS
26	10	4.4		TSBYG	72	28 d
27	10	4.4	Poised with citric acid	TSB	167	28 d
28	10	4.6	Poised with lactic acid	TSB	167	28 d
29	28	4	6 [5.12]% acetic acid	BHI	40	62 d
30	28	4	9 [3.78]% lactic acid	BHI	40	62 d
31	30	4	0.029% citric acid	TSBYE	51	42 d
32	30	4.5	0.068 [0.043]% acetic acid	TSBYE	51	42 d
33	30	4.5	0.043 [0.008]% lactic acid	TSBYE	51	42 d

Note: NS = Not stated.

[a] These are responsible for the deviation of the data points from the growth boundary predicted by the model.
[b] The following matrices refer to commonly used microbiological growth media: TSBYG; Tryptose-phosphate broth; Tryptose broth; TSBYE; TSB; Tryptic meat broth; BHI.
[c] Time for which no growth was observed.
[d] Concentration of acetic and lactic acids expressed as total, with undissociated in square brackets.

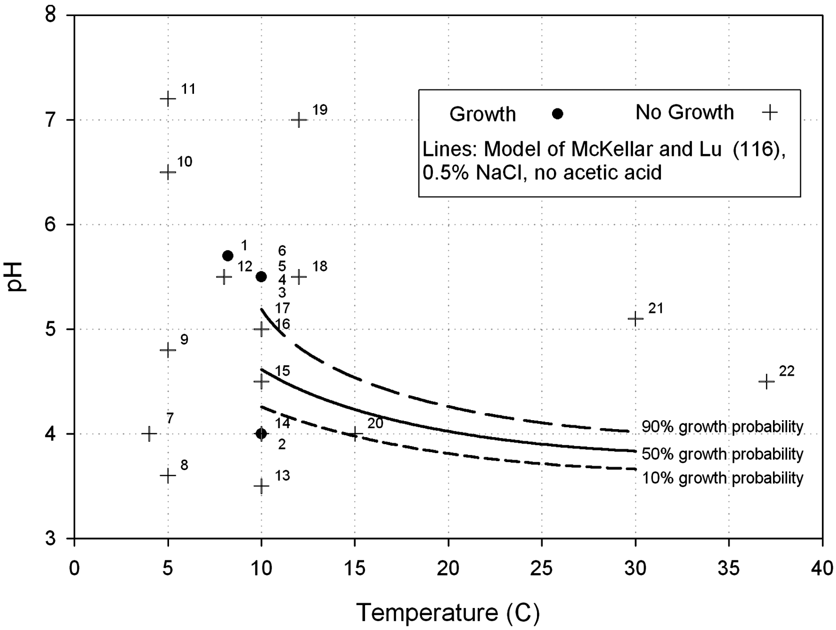


FIGURE 5.3 The growth boundary predicted by the model of McKellar and Lu[116] that predicts the growth limits of *Escherichia coli* O157:H7 as a function of temperature, pH, NaCl, sucrose, and acetic acid. This boundary is plotted alongside the data from the literature described in Table 5.4.

Dynamic modeling has also been validated,[25] where predictions from FoodMicroModel have been applied to the growth of *L. monocytogenes* and *Salmonella* in a range of foods incubated under constant as well as fluctuating temperatures. The authors found that generally the accuracy of prediction under the fluctuating temperatures was similar to the isothermal conditions, although inhibition by natural microflora did decrease the expected growth of *L. monocytogenes* in milk. Significant deviation of predictions of the growth of bacteria growing as colonies when immobilized in gel occurred when predictions were made from isothermal growth in broth.[125,126]

Validation of combined growth of the spoilage bacteria *Pseudomonas, Shewanella putrefaciens, Brochothrix thermosphacta,* and lactic acid bacteria was made in modified atmosphere packaged fish as a function of temperature and concentration of carbon dioxide.[91] Combined models based on polynomial, Belehradek, and Arrhenius equations were developed and validated by comparison with experimental growth rates of these bacteria obtained on three Mediterranean fish species. Predictions of the models based on the Belehradek and Arrhenius equations were judged satisfactory overall. This approach has been modified[90] to determine a procedure for modeling the shelf life of fish. Similarly, a quadratic response surface model has been used to describe the maximum specific growth rate of *Y. enterocolitica*. The model predicted growth rates as a function of refrigeration temperature and

TABLE 5.4
Literature Values Used in the Validation of the Growth Boundary Model Shown in Figure 5.3

Data Ref.	Temp. (°C)	pH	Other Hurdles[a]	Matrix[b]	Ref.	Obs. Time[c]
			***E. coli* — Growth Data**			
1	8.2	5.7		Ground mutton	83	
2	10	4	0.5% NaCl	TSB	116	
3	10	5.5	Poised with lactic acid	TSBYE	52	
4	10	5.5	Poised with citric acid	TSBYE	52	
5	10	5.5	0.5% NaCl	BHI	37	
6	10	5.5	5% NaCl	BHI	41	
			***E. coli* — No Growth Data**			
7	4	4		TSBYE	52	21 d
8	5	3.6		"Condiments"	183	7 d
9	5	4.8	a_w = 0.99	TSB	156	48 h
10	5	6.5	5% NaCl	BHI	41	12 d
11	5	≈7.2		Cucumber slices	1	10 d
12	8	5.5	0.5% NaCl	BHI	37	10 d
13	10	3.5	0.5% NaCl	TSB	116	72 h
14	10	4	Poised with acetic acid	TSBYE	52	21 d
15	10	4.5	5% NaCl	BHI	41	12 d
16	10	5	Poised with lactic acid	TSBYE	52	21 d
17	10	5	Poised with citric acid	TSBYE	52	21 d
18	12	5.5	30% sucrose, a_w = 0.972	BHI	36	24 h
19	12	≈7		Shredded carrot	1	10 d
20	15	4	0.5% NaCl	TSB	116	72 h
21	30	5.1	0.1 [0.03]% acetic acid as vinegar[d]	Nutrient agar	68	4 d
22	37	4.5	Poised with lactic acid	TSBYE	74	14 d

Note: NS = Not stated.

[a] These are responsible for the deviation of the data points from the growth boundary predicted by the model.

[b] The following matrices refer to commonly used microbiological growth media: TSBYE; TSB; BHI; Nutrient agar.

[c] Time for which no growth was observed.

[d] Concentration of acetic acid expressed as total, with undissociated in square brackets.

modified atmosphere and comparisons of the model predictions were made with growth rates obtained in seafood deliberately inoculated with *Y. enterocolitica.*[17]

Validations of the growth of *L. monocytogenes* in tryptose phosphate broth and in chicken and in beef have been made as a function of changing the pH and sodium chloride concentration.[133] Predictions of the growth of *L. monocytogenes* were then made using either a square root model[148] or a response surface polynomial model.[42] The square root model predicted growth rates at between 0 and 25°C with a

coefficient of determination of between 98.36 and 99.63%. The response surface polynomial model, however, predicted generation times at 5 to 25°C with between 0 and 17.4% difference between the observed and expected generation times in broth. Of greater significance in terms of validation in food here are the large differences observed in the generation time at pH 5.6 and 8°C (25.5 h) and the generation time predicted by the Pathogen Modeling Program (PMP) in these conditions in tryptose phosphate broth (5.3 h). The PMP is a web-based package developed in the U.S. that contains secondary models of the effects of environmental factors (mainly pH, concentration of NaCl, and temperature) on the survival, growth, and inactivation of major food-borne pathogenic bacteria in broth. A divergence from predicted values was also shown at temperatures between 0 and 3.5°C in the square root model.

Predictions of the growth of *Bacillus cereus* from PMP were validated for its growth from spores in boiled rice.[114] An analysis of variance showed that there was no statistically significant difference between the observed and measured growth rates in boiled rice and predictions made from PMP. Modeled predictions were fail-safe for generation time and exponential growth rate at all temperatures. Although the model was fail-safe for lag phase duration at 20 and 30°C, it was not at 15°C.

Modeling the growth of filamentous fungi is rare. The growth of three strains of heat-resistant fungi, as influenced by water activity adjusted using sucrose was modeled using the Baranyi model[13] to fit the changing colony diameter.[185] Modeling the growth of filamentous fungi has also been done using a model derived from the cardinal model family. The model was successfully fitted on data sets from a range of filamentous fungi whose growth was affected by a range of humectants including sodium chloride, glucose/fructose as a mixture, and glycerol and at different pH values. Further cardinal values were extracted from the literature and the model was used to predict the evolution of the radial growth of *Penicillium rocqueforti* and *Paecilomyces variotii*.[162]

In spite of the effort expended to develop and validate models, it is rare to find a model developed in broth that accurately predicts behavior in food systems. Models tend to fail-safe, and provide somewhat conservative predictions.[23,54,73,81,100,114,129,170,172] Indeed, the use of faster-growing strains has been suggested to provide a margin of safety.[123,131,194] Although many validations of models show that there is a fail-safe tendency and hence a margin of safety in growth prediction, some manufacturers of foods find that the error is unacceptable and the margin of safety provided by such models may well be more conservative than is desirable for many food applications. There are, however, examples of situations where the model makes what are clearly unsafe predictions, and these usually involve an overestimation of the extent of lag time.[69,188–190]

An alternative approach is to develop models directly in food products. This is not possible in many cases, due to the requirement of appropriate facilities for incorporating pathogens into the process under carefully controlled conditions. In spite of this limitation, models have been developed for growth of *L. monocytogenes* on vacuum-packed cooked meats[63] and liver pâté;[69] inactivation of *Salmonella typhimurium* in reduced calorie mayonnaise;[121] inactivation of *Enterobacteriaceae* and clostridia[18] and growth of *Lactobacillus* spp. in dry fermented sausage;[19] growth of

Clostridium botulinum in processed cheese;[169] thermal inactivation of *L. monocytogenes*[145] and *Enterococcus faecium*[161] during high-speed short-time pasteurization; and the thermal inactivation of *E. faecium* during cooking of Bologna sausage.[208] These models generally provide good estimates of the behavior of foodborne pathogens in food processes. However, it is questionable if effort should be expended developing models specific for all food processes. Improved validation techniques for models derived in broth or other model systems would appear to have more general applicability.

It has been suggested that models should only be regarded as first estimates of the behavior of pathogens, and that additional studies with products giving poor predictions should be undertaken.[195] Inclusion of additional data into models will often improve their predictive ability[207]; however, it is important that users of these models take great care in their use, and ensure that predictions are carefully validated in any product of concern.

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